

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

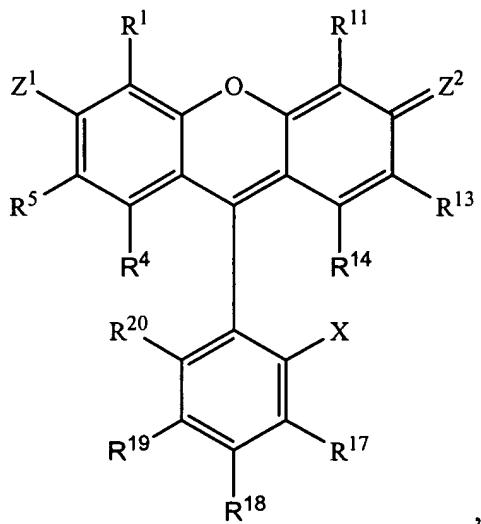
Listing of Claims:

1. (Cancelled)

2-55. (Previously Cancelled)

56. A method of forming a labelled substrate comprising:

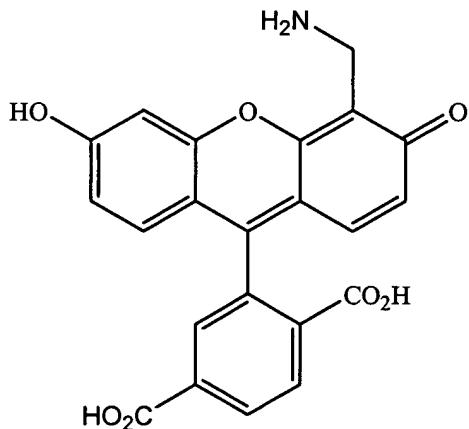
reacting a substrate selected from a polynucleotide, a nucleotide, a nucleoside, a polypeptide, a carbohydrate, a ligand, a substantially enantiomerically pure compound, a particle, and a surface, with the linking moiety of a substantially pure atropisomer compound having the structure:



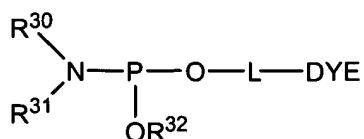
wherein Z¹ and Z² are each independently selected from O, OH, NH₂, NHR, and NR₂, X is selected from carboxylate and sulfonate; and at least one of R¹, R⁴, R⁵, R¹¹, R¹³, R¹⁴, R¹⁷, R¹⁸, R¹⁹, R²⁰, Z¹, or Z² is a linking moiety selected from azido, monosubstituted primary amine, disubstituted secondary amine, thiol, hydroxyl, halide, epoxide, N-hydroxysuccinimidyl ester, carboxyl, isothiocyanate, sulfonyl chloride, sulfonate ester, silyl halide, chlorotriazinyl, succinimidyl ester, pentafluorophenyl ester, maleimide, haloacetyl, epoxide, alkylhalide, allyl halide, aldehyde, ketone, acylazide, anhydride, iodoacetamide, phosphoramidite and an activated ester,

whereby a labelled substrate is formed.

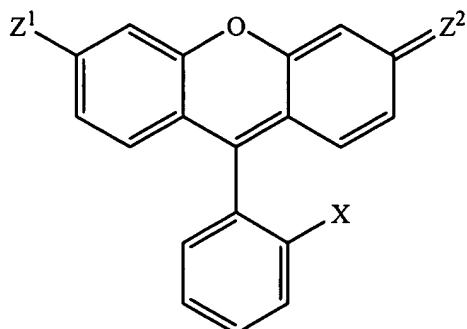
57. The method of claim 56 wherein the linking moiety is N-hydroxysuccinimide.
58. The method of claim 56 wherein the linking moiety is a phosphoramidite.
59. The method of claim 56 wherein the substrate is substantially enantiomerically pure.
60. The method of claim 56 wherein the substantially enantiomerically pure compound is (+)-menthyl chloroformate or (-)-menthyl chloroformate.
61. The method of claim 56 wherein the labelled substrate comprises C-11 aminomethyl, C-19 carboxyl fluorescein having the structure:



62. The method of claim 56 wherein the particle is a nanoparticle, a microsphere, a bead, or a liposome.
63. The method of claim 56 wherein the surface is glass.
64. A method of synthesizing a labelled polynucleotide comprising:
coupling a phosphoramidite compound of the structure:



wherein DYE is a substantially pure atropisomer of a xanthene compound having the structure:



and aryl-substituted forms thereof,

wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate;

L is a linker;

R^{30} and R^{31} taken separately are selected from the group consisting of C₁-C₁₂ alkyl, C₁-C₁₂ cycloalkyl, and aryl; or R^{30} and R^{31} taken together with the nitrogen atom form a saturated nitrogen heterocycle; and

R^{32} is a phosphite ester protecting group,

to a polynucleotide, wherein the polynucleotide is bound to a solid support, whereby a labelled polynucleotide is formed.

65. A method of separating atropisomers of a C-11 aminomethyl, C-19 carboxyl fluorescein compound comprising:

- (a) reacting a C-11 aminomethyl, C-19 carboxyl fluorescein with a substantially pure enantiomer of an active ester or carboxylic acid to form diastereomeric carbamates;
- (b) separating the diastereomeric carbamates; and
- (c) hydrolyzing the separated diastereomers with aqueous acid.

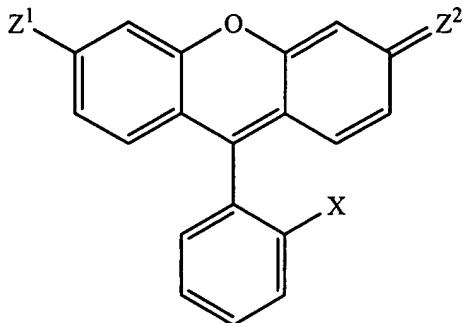
66. The method of claim 65 wherein the active ester is menthyl chloroformate.

67. The method of claim 65 wherein the diastereomeric carbamates are separated by reverse-phase HPLC.

68. A method of separating a mixture of labelled substrates comprising:

- (a) separating a mixture of labelled substrates by electrophoresis; and
- (b) detecting the labelled substrates by fluorescence detection,

wherein the labelled substrates are comprised of a substantially pure atropisomer of a xanthene compound having the structure:



and aryl-substituted forms thereof,

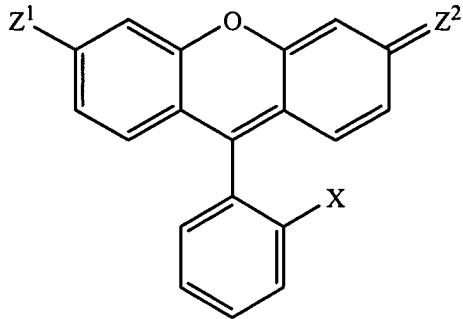
wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate.

69. The method of claim 68 wherein the labelled substrates are labelled polynucleotides.

70. A method of separating a mixture of labelled substrates comprising:

- (a) separating a mixture of labelled substrates by chromatography; and
- (b) detecting the labelled substrates by fluorescence detection,

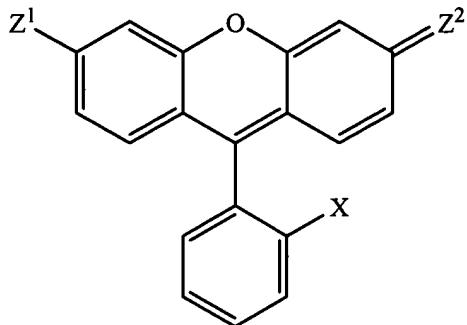
wherein the labelled substrates are comprised of a substantially pure atropisomer of a xanthene compound having the structure:



and aryl-substituted forms thereof,

wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate.

71. A method of generating a labelled primer extension product, comprising the step of extending a primer-target hybrid with an enzymatically-incorporatable nucleotide, wherein said primer or said nucleotide is labelled with a substantially pure atropisomer of a xanthene compound having the structure:



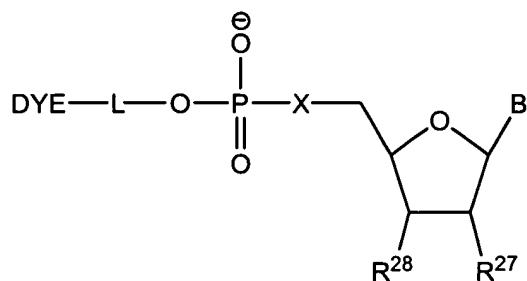
and aryl-substituted forms thereof,

wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate,

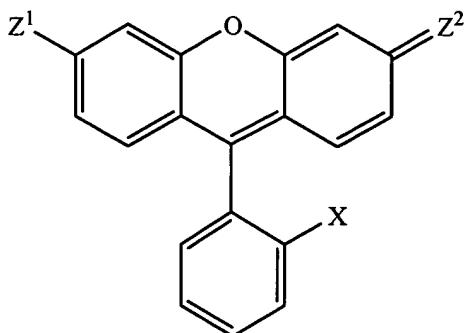
whereby the primer is extended.

72. The method of claim 71 wherein the nucleotide is enzymatically-extendable.

73. The method of claim 71 wherein the primer is a labelled polynucleotide comprising the formula:



wherein DYE is a substantially pure atropisomer of a xanthene compound having the structure:



and aryl-substituted forms thereof,

wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate;

B comprises a nucleobase;

X is selected from O, NH and S;

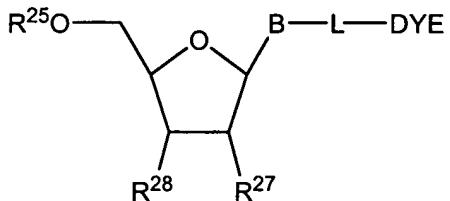
L comprises a linker;

R^{27} is selected from H, OH, halide, azide, amine, alkylamine, C₁-C₆ alkyl, allyl, C₁-C₆ alkoxy, OCH₃, and OCH₂CH=CH₂; and

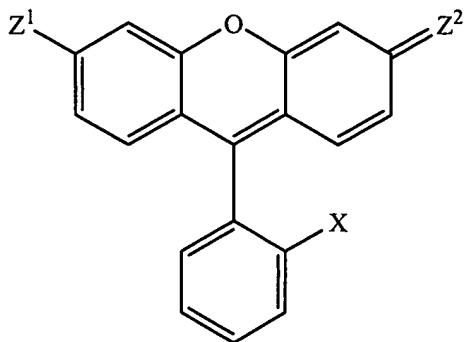
R^{28} is selected from an internucleotide phosphodiester and an internucleotide analog; wherein the polynucleotide comprises 2 to 82 nucleotides.

74. The method of claim 73 wherein B is selected from the group consisting of uracil, thymine, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanosine.

75. The method of claim 71 wherein the enzymatically-incorporatable nucleotide is a labelled nucleoside or nucleotide having the formula:



wherein DYE is a substantially pure atropisomer of a xanthene compound having the structure:



and aryl-substituted forms thereof,

wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate;

B comprises a nucleobase;

L comprises a linker;

R^{25} is selected from H, monophosphate, diphosphate, triphosphate, thiophosphate, and phosphate analog; and

R^{26} and R^{27} are each independently selected from -H, -OH, -F and a moiety which blocks polymerase-mediated target-directed primer extension.

76. The method of claim 71 further comprising a terminator nucleotide.

77. The method of claim 75 wherein R^{26} and R^{27} are -H.

78. A method of polynucleotide sequencing comprising:

a) forming a mixture of a first, a second, a third, and a fourth class of polynucleotides, such that:

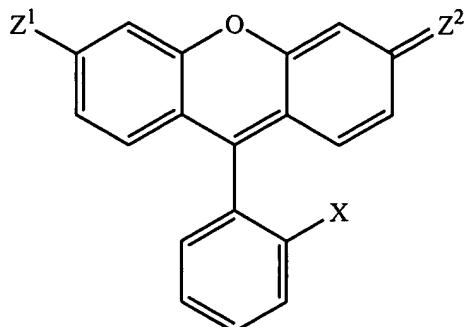
each polynucleotide in the first class includes a 3'-terminal dideoxyadenosine and is labelled with a first dye;

each polynucleotide in the second class includes a 3'-terminal dideoxycytidine and is labelled with a second dye;

each polynucleotide in the third class includes a 3'-terminal dideoxyguanosine and is labelled with a third dye; and

each polynucleotide in the fourth class includes a 3'-terminal dideoxythymidine and is labelled with a fourth dye;

wherein at least one of the first, second, third, or fourth dyes is a substantially pure atropisomer of a xanthene compound having the structure:



and aryl-substituted forms thereof,

wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate, and the other dyes are spectrally resolvable from each other; and

b) separating the polynucleotides on the basis of size.

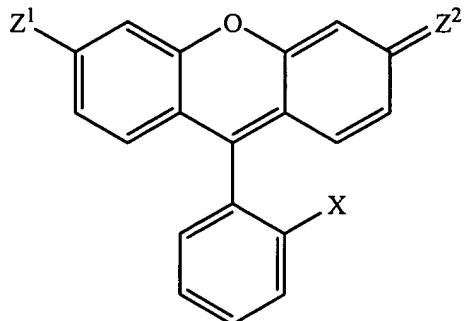
79. The method of claim 78 further comprising the step of detecting the separated polynucleotides by fluorescence detection.

80. The method of claim 78 further comprising the step of identifying the 3'-terminal nucleotide of the polynucleotides by the fluorescence spectrum of the dyes.

81. A method of oligonucleotide ligation, comprising:

annealing two probes to a target sequence and forming a phosphodiester bond between the 5' terminus of one probe and the 3' terminus of the other probe;

wherein one or both probes are labelled with a substantially pure atropisomer of a xanthene compound having the structure:



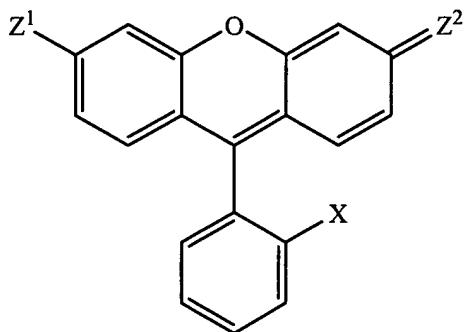
and aryl-substituted forms thereof,

wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate.

82. A method of fragment analysis comprising:

separating labelled polynucleotide fragments by a size-dependent separation process; and detecting the separated labelled polynucleotide fragments subsequent to the separation process,

wherein the fragments are labelled with a substantially pure atropisomer of a xanthene compound having the structure:



and aryl-substituted forms thereof,

wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate.

83. The method of claim 82 wherein the fragments are labelled with a mobility-modifying label.

84. The method of claim 82 wherein the fragments are formed by ligation.

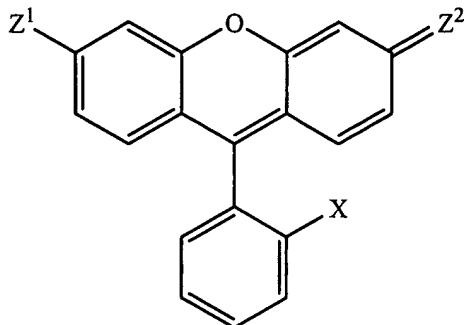
85. The method of claim 82 wherein the size-dependent separation process is electrophoresis and the labelled polynucleotide fragments are detected by fluorescence.

86. A method of amplification comprising:

annealing two or more primers to a target polynucleotide; and

extending the primers by a polymerase and a mixture of enzymatically-extendable nucleotides;

wherein at least one of the primers is a labelled polynucleotide comprising a polynucleotide covalently attached to a label, wherein the label is a substantially pure atropisomer of a xanthene compound having the structure:



and aryl-substituted forms thereof,

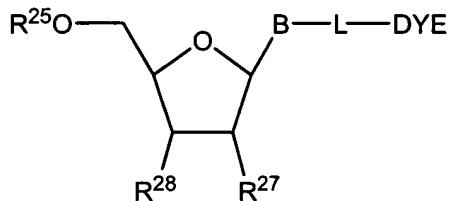
wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate.

87. A method of amplification comprising:

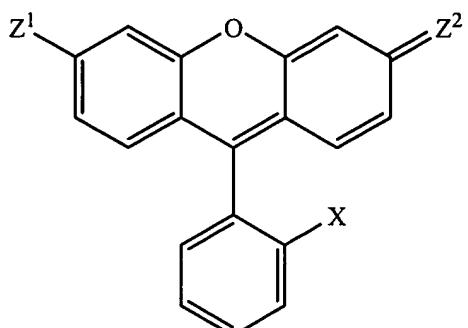
annealing two or more primers to a target polynucleotide, and

extending the primers by a polymerase and a mixture of enzymatically-extendable nucleotides;

wherein at least one of the nucleotides is a labelled nucleotide having the formula:



wherein DYE is a substantially pure atropisomer of a xanthene compound having the structure:



and aryl-substituted forms thereof,

wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate;

B comprises a nucleobase;

L comprises a linker;

R^{25} is selected from monophosphate, diphosphate, triphosphate, thiophosphate, and phosphate analog; and

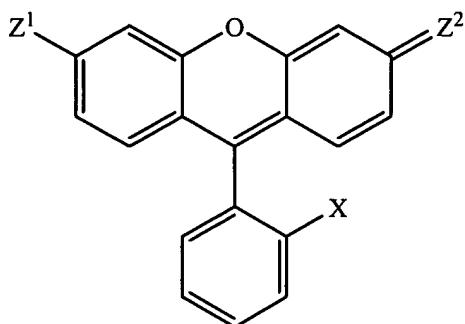
R^{26} and R^{27} , when taken alone, are each independently selected from -H, -OH, -F and a moiety that blocks polymerase-mediated target-directed primer extension.

88. A method of amplification comprising:

annealing two or more primers and a fluorescent dye-quencher probe to a target nucleic acid; and

extending the primers by polymerase and a mixture of enzymatically-extendable nucleotides;

wherein the probe is a labelled polynucleotide comprising a polynucleotide covalently attached to a label, wherein the label is a substantially pure atropisomer of a xanthene compound having the structure:



and aryl-substituted forms thereof,

wherein Z^1 and Z^2 are each independently selected from O, OH, NH₂, NHR and NR₂, X is carboxylate or sulfonate.